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XAS Studies of Coordination Complexes Modeling the Structure and Reactivity of Lipoxygenases

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Beamlines: X18b and (in previous years) X9b

Introduction: Lipoxygenases catalyze the reaction of polyunsaturated fatty acids with molecular oxygen to give fatty acid hydroperoxides. The generally accepted mechanism involves H-atom abstraction from the fatty acid by an iron(III)-hydroxide group. Earlier EXAFS and XANES studies, performed at beam line X9 at the NSLS (reference below), supported this mechanism by showing the presence of a ca. 1.9Å Fe(III)-O bond in the active form of soybean lipoxygenase-1; this distance is shorter than expected for bonds to any amino acid sidechains at the active site of lipoxygenase, but is expected for a hydroxide ligand. Crystallographic studies have revealed that the other ligands to the iron include three or four (depending on species) histidine sidechains and the carboxylate group from the C-terminus of the polypeptide.

Methods and Materials: We are now engaged in a study of the spectroscopy and reactivity of inorganic complexes with ligands that mimic the active site of lipoxygenases. One such ligand is N'-benzyl-N,N'-bis(2-pyridylmethyl)-ethylenediamine N-acetic acid; because it can coordinate to iron with four nitrogen and one oxygen atoms, we denote this ligand (in its neutral acid form) as HL^{N4O}. When the iron(II) complex of this ligand ($FeL^{N4O}Cl$) is reacted with *t*-butylhydroperoxide (*t*BuOOH) in a methanol/water solvent system, a mononuclear iron(III) complex is formed in solution; ESR spectroscopy and electrospray mass spectrometry both support the formulation of this species as $[FeL^{N4O}OH]^+$. To further confirm this formulation and compare the iron coordination in this species to that in soybean lipoxygenase-1, we reacted ca. 10 mM $FeL^{N4O}Cl$ (in a 4:1 MeOH/H_2O solvent system) with a stoichiometric excess of *t*BuOOH at room temperature. The sample was frozen in approximately 1 minute after mixing, and X-ray absorption spectra were obtained using a Si111 monochromator, a Canberra 13-element solid state fluorescence detector and a helium displex cryostat to maintain the sample at ca. 100 K.

Results: The Fe K-edge spectra show about a 2 eV positive shift in edge energy caused by treatment of $FeL^{N4O}Cl$ with *t*BuOOH, confirming the change of oxidation state of the iron from +2 to +3. The figure below shows that the EXAFS spectrum of the frozen solution of the oxidized compound is very similar to that obtained previously of the active form of soybean lipoxygenase-1. Also, the observed spectra rule out the formation of a μ-oxo diferric species under the millimolar solution concentrations used in the EXAFS experiment, since a different spectrum (analyzing for a shorter Fe-O bond length) is obtained on a solid state sample of the dimeric species.

Conclusions: The X-ray absorption spectroscopy supports the results from EPR and mass spectrometry that the oxidation product is $[FeL^{N4O}OH]^+$. This system models the formation of the active form of lipoxygenase by treatment of the reduced (iron(+2)) form of the enzyme with lipid hydroperoxides.

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Reference: R. C. Scarrow, M. G. Trimitsis, C. P. Buck, G. N. Grove, R. A. Cowling, and M. J. Nelson, "X-ray Spectroscopy of the Iron Site in Soybean Lipoxygenase-1: Changes in Coordination upon Oxidation or Addition of Methanol," Biochemistry, **1994**, *33*, 15023-15035.